[ ] a substitute computer readable form to replace one found to be damaged or unreadable.

[ ] The computer readable form in this application no. 09/... is identical with that filed on .... [date sequence was filed] in application no. 09/ , filed [filing date]. In accordance with 37 C.F.R. §1.821(e), please use the [first-filed, last-filed or only, whichever is applicable] computer readable form filed in that application as the computer readable form for the instant application. It is understood that the Patent and Trademark Office will make the necessary change in application number and filing date for the instant application. A paper copy of the Sequence Listing is [included in the originally-filed specification of the instant application, included in a separately filed preliminary amendment for incorporation into the specification, whichever is applicable].

[XX] 2. The description has been amended to comply with §1.821(d).

3. The undersigned attorney or agent hereby states as follows:

(a) this submission is not believed to include new matter [§1.821(g)];

(b) the contents of the paper copy (as amended, if applicable) and the computer readable form of the Sequence Listing, are believed to be the same [§1.821(f) and §1.825(b)];

(c) if the paper copy has been amended, the amendment is believed to be supported by the specification and is not believed to include new matter [§1.825(a)]; and

(d) if the computer readable form submitted herewith is a substitute for a form found upon receipt by the PTO to be damaged or unreadable, that the substitute data is believed to be identical to that originally filed [§1.825(d)].

4. We have some specific comments to make concerning Figure 9, on page 19. Figure 9B refers to sequences identified as "FMK1" and as "ERK1". We wish to point out for the record that these correspond to F. oxysporum AF 286533 (EMBL) and C. albicans P 28869 (Swiss Prot), respectively. Likewise Figure 9A refers to "KLAC" and "CPUR", and these correspond to K. lactis Q08400 (Swiss Prot) and C.

lusitaniae, AF 175524 (EMBL). We have inserted SEQ ID NOs, but we have not added database citations.

In addition, we would like to direct the Examiner's attention to SEQ ID NO:1 of the Sequence Listing filed as part of the original application papers. On the line ending "842", Pro is said to be encoded by "cc" (bases 796-797). It is of course necessary that Pro be encoded by a triplet, not a doublet; this triplet can be "cca", "ccg", "cct", or "ccc". It is clear that the third base has been omitted, not merely misaligned", since "Asn" is encoded by "aat", the next codon, whereas if the first "a" of "aat" were transferred to "cc" (forming a "cca" Pro codon), the next codon "atg" would encode Met.

It follows that a base, between the "cc" for Pro and the "aat" for Asn, was omitted. It could be any of the four bases since all four possible triplets encode Pro. Consequently, we have amended SEQ ID NO:1 by inserting as base 798 the letter "n", which represents all four bases. This does not add new matter as a Pro codon was already called for and "ccn" encodes Pro.

As a result, the second exon was 796-1707 and the third exon was 1761-1928. The last intron was consequently 1708-1760.

4. Under U.S. rules, each sequence must be classified in <213> as an "Artificial Sequence", a sequence of "Unknown" origin, or a sequence originating in a particular organism, identified by its scientific name.

Neither the rules nor the MPEP clarify the nature of the relationship which must exist between a listed sequence and an organism for that organism to be identified as the origin of the sequence under <213>.

Hence, counsel may choose to identify a listed sequence as associated with a particular organism even though that sequence does not occur in nature by itself in that organism (it may be, e.g., an epitopic fragment of a naturally occurring protein, or a cDNA of a naturally occurring mRNA, or even a substitution mutant of a naturally occurring sequence). Hence, the identification of an organism in <213> should not be construed as an admission that the sequence *per se* occurs in nature in said organism.

Similarly, designation of a sequence as "artificial" should not be construed as a representation that the sequence has no association with any organism. For example, a primer or probe may be designated as "artificial" even though it is necessarily complementary to some target sequence, which may occur in nature. Or an "artificial" sequence may be a substitution mutant of a natural sequence, or a chimera of two

or more natural sequences, or a cDNA (i.e., intron-free sequence) corresponding to an intron-containing gene, or otherwise a fragment of a natural sequence.

The Examiner should be able to judge the relationship of the enumerated sequences to natural sequences by giving full consideration to the specification, the art cited therein, any further art cited in an IDS, and the results of his or her sequence search against a database containing known natural sequences.

Attached hereto is a marked-up version of the changes made to the specification by the current amendment. The attached page is captioned "Version with markings to show changes made".

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

The paragraph beginning at line 24 of page 17 has been amended as follows:

Features of the M. circinelloides *pkaR* promoter (1-600 of SEQ ID NO:1): Putative CAAT boxes are shown in uppercase; a putative TATA box and a CT-rich stretch are depicted underlined; the start of the coding region (1-19 of SEQ ID NO:2) is shown in uppercase with the translated protein in one-letter code.

The paragraph beginning at line 30 of page 17 has been amended as follows:

Multiple alignment of PKARs. The M. circinelloides PKAR was aligned with other fungal PKAR sequences. Identical residues are boxed. Abbreviations and accession numbers: Mcir: M. circinelloides, AJ400723 (EMBL) (SEQ ID NO:36); Anig: Aspergillus niger, Q9C196 (SwissProt) (SEQ ID NO:37); Beme: Blastocadiella emersonii, P31320 (SwissProt) (SEQ ID NO:38); Calb: C. albicans Q9HEW1 (SwissProt) (SEQ ID NO:39); Scer: S. cerevisiae, P07278 (SwissProt) (SEQ ID NO:40), Spom: Schizosaccharomyces pombe, P36600 (SwissProt) (SEQ ID NO:41); Mrou: M. rouxii, Q9P8K6 (SwissProt) (SEQ ID NO:42).

The paragraph beginning at line 5 of page 18 has been amended as follows:

Alignment of M. circinelloides PKAC with other fungal counterparts. Identical residues are boxed. Abbreviations and accession numbers: M.cir: M. circinelloides, ~~AJ400723~~ AJ431364 (EMBL) (SEQ ID NO:43); Anig: A. niger, P87077 (SwissProt) (SEQ ID NO:44); Beme: B. emersonii, Q12741 (SwissProt) (SEQ ID NO:45); Calb: C. albicans Q9HEW0 (SwissProt) (SEQ ID NO:46); Scer: S. cerevisiae, P06245 (SwissProt) (SEQ ID NO:47), Spom: S. pombe, P40376 (SwissProt) (SEQ ID NO:48).

The paragraph beginning at line 23 of page 18 has been amended as follows:

Overexpression of PKAR in M. circinelloides. A: Plasmid map (left) of pEUKA4-pkaR. Northern blot analysis (middle panel) of KFA121 (a pEUKA4-pkaR transformant) grown in YNB medium with 5 % glucose (lane 2). The same conditions were used for the control strain KFA89 (lane 1). The RNA gel is shown below for loading control. Primer extension analysis (right panel): the fragment obtained is indicated with an arrow; a sequence ladder was run on pEUKA4-pkaR to determine the transcription start site (tss). The sequence obtained (SEQ ID NO:49) is shown below (the arrow indicates the tss; mRNA sequence is shown in italics, cloning site (*XhoI*) and ATG start codon of *pkaR* (bold). B: Colony morphology of KFA121

(right) and KFA89 (left) on YNB plates (2 % glucose) showing the higher branching degree of KFA121.

The paragraph beginning at line 6 of page 19 has been amended as follows:

The M. circinelloides STE12 and MPK1 homologues. A: The protein sequence corresponding to the identified M. circinelloides *ste12* fragment was aligned with relevant fungal STE12 sequences. Abbreviations and accession numbers: Mcir: M. circinelloides, AJ400723~~3~~4 (EMBL) (SEQ ID NO:50); A.nid: A. nidulans, O74252 (SwissProt) (SEQ ID NO:54); Calb: C. albicans P43079 (SwissProt) (SEQ ID NO:52); Scer: S. cerevisiae, P13574 (SwissProt) (SEQ ID NO:51); Klac (SEQ ID NO:53); Cpur (SEQ ID NO:55). B: The protein sequence corresponding to the identified M. circinelloides *mpk1* fragment (MPK1) was aligned with relevant fungal STE12 sequences. Abbreviations and accession numbers: Mcir: M. circinelloides, ~~AJ400723~~ AJ309731 (EMBL) (SEQ ID NO:56); Calb (MKC1): C. albicans P43068 (SwissProt) (SEQ ID NO;58); Scer (SLT2): S. cerevisiae, Q00772 (SwissProt) (SEQ ID NO:60), Spom (SPM1): S. pombe, Q92398 (SwissProt) (SEQ ID NO:57); FMK1 (SEQ ID NO:59); ERK1 (SEQ ID NO:61).

The paragraph beginning at line 18 of page 19 has been amended as follows:

Nucleotide sequence (SEQ ID NO:24) and derived amino-acid sequence (SEQ ID NO:25) of *gpd1*. Numbering of nucleotides is with respect to the start of the coding sequence. Exon sequences are capitalised. Sequences with homology to the lariat formation consensus sequence within introns are italicised. Putative TATA and CAAT boxes are boxed and bolded, respectively. Pyrimindine stretch is underlined. The putative polyadenylation signal is double underlined. The transcription start point is capitalised and bolded. The sequence corresponding to the gene-specific oligonucleotide used in Northern blotting and primer extension is wavy underlined.

The paragraph beginning at line 19 of page 47 has been amended as follows:

Many putative downstream effectors of the small GTPases Cdc42 and Rac contain a GTPase binding domain (GBD), also called p21 binding domain (PBD), which has been shown to specifically bind the GTP bound form of Cdc42 or Rac, with a preference for Cdc42. The most conserved region of GBD/PBD domains is the N-terminal Cdc42/Rac interactive binding motif (CRIB), which consists of about 16 amino acids with the consensus sequence I-S-X-P-X(2,4)-F-X-H-X(2)-H-V-G (SEQ ID NOs:62-63). Although the CRIB motif is necessary for the

binding to Cdc42 and Rac, it is not sufficient to give high-affinity binding.

The paragraph beginning at line 30 of page 51 has been amended as follows:

The term catalytic domain as used herein above shall include the conserved TXY motif in which both the threonine and tyrosine residues are phosphorylated during activation of the enzyme by upstream dual-specificity MAP kinase kinases (MAPKKs). In addition to the TXY motif, other motifs include the region located just after the TXY motif and containing a F and a C residue that are MAPK-specific. The R and E residues in the first part of the pattern, and the R, D and K residues in the second part, are shared by many additional protein kinases. They have been included in the pattern to eliminate matches from unrelated sequences in the database, and to "anchor" the MAPK-specific F and C residues to this region. Accordingly, one preferred catalytic domain comprises the consensus pattern: F-x(10)-R-E-x(72,86)-R-D-x-K-x(9)-C (SEQ ID NOs:64-65), and this domain is preferably recognised by an antibody used to define fragments of MAPK in accordance with the present invention.

The paragraph beginning at line 8 of page 120 has been amended as follows:

The protein sequences of several fungal regulatory subunits of protein kinase A (PKAR) are present in the public databases. The high level of sequence homology allowed the design of degenerate primers (Table 1) derived from the GDFFYVVE and WALDRNTS regions (positions 21~~9~~3-22~~6~~0 and 2~~7~~266-27~~9~~3 in the M. circinelloides PKAR protein sequence (SEQ ID NO:2), see below) and the PCR amplification of a 183-bp fragment, named pkaR13b-1. Sequence analysis and database searches identified pkaR13b-1 as highly homologous to known fungal and eukaryotic PKAR encoding genes. Using pkaR13b-1 as a probe, a positive clone, pkaR1, was identified from a M. circinelloides genomic library. Sequence analysis of the 2-kb insert in pkaR1 showed that it contained a chromosomal insert encompassing the full-length M. circinelloides *pkaR* gene including 40-bp upstream of the ATG start codon. Further cloning using inverse PCR allowed the characterisation of the upstream region of *pkaR* including 541 bp of the promoter region (Fig. 3).

The paragraph beginning at line 14 of page 121 has been amended as follows:

The M. circinelloides PKAR displays an overall homology to other fungal PKARs (31-45 % identity, Fig. 4) and contains the expected well-conserved domains. Thus, two domains with a high degree of homology to cAMP-binding domains

in other PKARs (94 to 64 % identity), are present in the M. circinelloides PKAR (sFGELALmynAPRAATii and yFGELALlndAPRAATvv, at positions 247-264 and 369-386, respectively, in the amino acid sequence, Fig. 4). Further, a putative kinase inhibitor domain (RRTSVK) is found at position 144-149 in the amino acid sequence (SEQ ID NO:36) (Fig. 4). The partial sequence available from the PKAR of the related fungus M. rouxii does not include this domain (Sorol et al., 2000) and therefore comparison of the PKAR kinase inhibitor domain between these two Mucor species awaits.

The paragraph beginning at line 7 of page 122 has been amended as follows:

The *pkaC* gene contains two putative introns in the 5' end and putatively encodes a protein of 605 amino acid residues. This protein contains an ATP-binding domain (GQGSVG at position 254-259 in the amino acid sequence (SEQ ID NO:43), Fig. 5) and a region with high homology to a serine/threonine protein kinase active site (position 367-379 in the amino acid sequence). Surprisingly, the conserved active site aspartic acid residue found in other PKACs is in M. circinelloides replaced by an aspargine residue. The codon encoding the aspargine residue (AAC) was confirmed by sequencing of different independent PCR products. However, we cannot

completely rule out the possibility that this divergence is due to a PCR amplification artefact.

The paragraph beginning at line 3 of page 126 has been amended as follows:

STE12 is a transcription factor that participates in the MAPK-dependent signal transduction pathway in S. cerevisiae, C. albicans and C. neoformans leading to filamentation (Liu et al., 1994, Yue et al., 1999). In C. albicans, a *ste12* null mutant strain is defective in filamentation (Liu et al., 1994). The STE12 transcription factor consists of a N-terminal region involved in DNA binding, a central induction domain and a C-terminal region involved in transcriptional activation. To investigate whether M. circinelloides possesses a *ste12* homologue, PCR was carried out using degenerate primers designed from the most conserved sequence of the N-terminal region of available *ste12* genes (KFFLATA and QKKQKVF, positions 44-50 and 151-157 in the S. cerevisiae STE12 protein sequence (SEQ ID NO:51), Fig. 9A). A 384-bp fragment, ste12b-1, was obtained using R7B DNA as template. Sequence analysis revealed a high degree of homology between the protein sequence encoded by the ste12b-1 DNA sequence and other fungal STE12 homologues (56-64%, Fig. 9A), confirming that the cloned fragment is part of the M. circinelloides *ste12* gene.